# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA05/000099

International filing date: 28 January 2005 (28.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/540,804
Filing date: 30 January 2004 (30.01.2004)

Date of receipt at the International Bureau: 30 March 2005 (30.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PCT/CA 05000099
11 March 2005 (11.03.2005)

PA 1279348

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

**UNITED STATES DEPARTMENT OF COMMERCE**

**United States Patent and Trademark Office**

**February 02, 2005**

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.**

**APPLICATION NUMBER:** *60/540,804*
**FILING DATE:** *January 30, 2004*

**By Authority of the**
**COMMISSIONER OF PATENTS AND TRADEMARKS**

N. Woodson

**N. WOODSON**
**Certifying Officer**

Express Mail Label: EV392153621US
Date of Deposit: January 30, 2004

Attorney Docket No. 24492-019

# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

**This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)**



| INVENTORS / APPLICANTS | | | |
|---|---|---|---|
| Given Name (last name, first name, and middle initial [if any]) | | | Residence (City and State or Foreign Country) |
| Cruz | Antonio | | Toronto, Ontario, Canada |
| Pastrak | Aleksandra | | Toronto, Ontario, Canada |
| Hew | Yin | | Thornhill, Ontario, Canada |

**TITLE OF THE INVENTION**

COMBINED USE OF A GLP-1 AGONIST AND NOVEL GASTRIN COMPOUNDS

**CORRESPONDENCE ADDRESS**

| | |
|---|---|
| Attorney Name:<br>Firm Name and Address: | Sonia K. Guterman, Ph.D., J.D.<br>MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C.<br>One Financial Center<br>Boston, MA 02111 |
| Telephone: | (617) 542-6000 |
| Fax: | (617) 542-2241 |

**ENCLOSED APPLICATION PARTS**

- ☒ **Specification** — Number of Pages: 45
- ☐ **Sequence Listing** — Number of Pages:
- ☐ **Drawings** ( ☐ Formal; ☐ Informal) — Number of Sheets:
- ☐ **Other documents** (specify):

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government:

- ☒ No.
- ☐ Yes, the name of the U.S. Government agency and the Government contract number are:

**METHOD OF PAYMENT**

- ☒ A check in the amount of **$80.00** is enclosed to cover the filing fees of the Provisional application.
- ☐ The Commissioner is hereby authorized to charge **$80.00** is enclosed to cover the filing fees of the Provisional application to Deposit Account No. 50-0311, Reference No. 24492-019
- ☒ The Commissioner is hereby authorized to charge additional fees or credit any overpayment to Deposit Account No. 50-0311, Reference No. 24492-019.

Respectfully submitted,

Reg. No. 41,874

Date: January 30, 2004

Sonia K. Guterman, Esq., Reg. No. 44,729
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C.
One Financial Center
Boston, MA 02111
Telephone: (617) 542-6000
**Customer No. 30623**

TRA 1880283v1

**Title: Combined Use of a GLP-1 Agonist and Novel Gastrin Compounds**

## FIELD OF THE INVENTION

The invention relates generally to compositions, conjugates, and methods comprising a GLP-1 agonist and a gastrin compound, and uses thereof.

## BACKGROUND OF THE INVENTION

Glucagon-like peptide-1 (GLP-1) is a physiological incretin hormone from the lower gastrointestinal tract. GLP-1 has significant physiological activities including stimulation of glucose-dependent insulin secretion, inhibition of glucagon secretion and gastric emptying, inhibition of food intake, enhancement of glucose utilization, preservation of beta cells, inhibition of beta cell apoptosis, and induction of beta cell proliferation. [See Nauck, M.A. Acta Diabetol, 1998, 35:117-129; Holst J.J. Deabetes Metab Res Rev 2002, 18:430-441; Reimer, R.A. et al, Endocrinology 142(10): 4522-4528; Drucker, D.J. , Molecular Endocrinology, 2003, 17(2) 161-171 and http://www.glucagon.com for reviews of GLP-1.] The above stated activities of GLP-1 make it a highly desirable therapeutic agent for the treatment of many conditions and diseases including diabetes, obesity, gastric ulcers, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome, and related diseases and disorders.

The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

## SUMMARY OF THE INVENTION

The combination of a GLP-1 agonist and a gastrin compound provides beneficial effects in the treatment of conditions for which either a GLP-1 agonist or a gastrin compound have been demonstrated to have a therapeutic effect, including but not limited to diabetes, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases and disorders, and obesity. Combinations of a GLP-1 agonist and a gastrin compound may be selected to provide unexpectedly additive effects or greater than additive effects i.e. synergistic effects.

A composition, conjugate, or combination therapy comprising a GLP-1 agonist and a gastrin compound employing different mechanisms to achieve maximum therapeutic efficacy, may improve tolerance to the therapy with a reduced risk of side effects that may result from higher doses or longer term monotherapies (i.e. therapies with each compound alone). A composition or combination treatment of the invention will permit the use of

lower doses of each compound with reduced adverse toxic effects of each compound. A suboptimal dosage may provide an increased margin of safety, and may also reduce the cost of a drug necessary to achieve prophylaxis and therapy. In addition, the increased convenience of a single combination dosage unit will result in enhanced compliance.

Significantly, a composition, conjugate, or combination treatment of the invention provides sustained beneficial effects following treatment. Prolonged efficacy may be evidenced by increased C-peptide production, increases in pancreatic insulin production, and/or about normal blood glucose levels compared with GLP-1 or gastrin alone.

The invention contemplates a composition, preferably a pharmaceutical composition, comprising a GLP-1 agonist and a gastrin compound which provide beneficial effects relative to each compound alone. A pharmaceutical composition may optionally comprise a pharmaceutically acceptable carrier, excipient, or vehicle.

In an aspect the invention provides a pharmaceutical composition, comprising a GLP-1 agonist and a gastrin compound that provide beneficial effects, preferably sustained beneficial effects, following treatment. In an embodiment, the sustained beneficial effects are evidenced by one or more of the following: (a) an increase in C-peptide production, (b) an increase in pancreatic insulin production, and/or (c) about normal blood glucose levels compared with GLP-1 or gastrin alone.

The invention also provides a pharmaceutical composition in separate containers and intended for simultaneous or sequential administration to provide beneficial effects, preferably sustained beneficial effects, comprising a GLP-1 agonist and a gastrin compound, both optionally together with pharmaceutically acceptable carriers, excipients, or vehicles.

The invention also provides a pharmaceutical composition for the treatment of a disease or condition comprising a therapeutically effective amount of a GLP-1 agonist and a gastrin compound in a pharmaceutically acceptable carrier, excipient, or vehicle.

In another aspect the invention provides a combination treatment for treating or preventing a condition or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one GLP-1 agonist and a gastrin compound to provide beneficial effects. In an aspect the invention provides a combination treatment which provides sustained beneficial effects following treatment.

The invention provides a conjugate comprising a GLP-1 agonist linked to a gastrin compound to provide beneficial effects, preferably sustained beneficial effects described herein.

The invention also provides methods for preparing compositions and conjugates of the invention that result in compositions and conjugates with beneficial effects, preferably sustained beneficial effects.

The invention additionally provides a method of preparing a stable pharmaceutical composition of a GLP-1 agonist adapted to provide beneficial effects, preferably sustained beneficial effects, following treatment, comprising preparing a composition comprising the GLP-1 agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the GLP-1 agonist.

The invention also contemplates the use of a composition or conjugate of the invention or combination treatment of the invention for preventing, and/or ameliorating disease severity, disease symptoms, and/or periodicity of recurrence of a disease or condition described herein. The invention also relates to the prevention and treatment, in a subject, of diseases or conditions using the compositions, combination treatments, and conjugates of the invention.

The invention provides a method of treating a disease or condition comprising administering a GLP-1 agonist and a gastrin compound, a composition or conjugate of the invention with a plurality of cells to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects. In an embodiment, the compounds/composition/conjugate are administered systemically.

The invention also relates to a method for expanding and differentiating stem cells or progenitor cells into insulin secreting cells comprising contacting the stem cells or progenitor cells with a GLP-1 agonist and a gastrin compound or a composition or conjugate of the invention in sufficient amounts to expand and differentiate stem cells or progenitor cells. The amount of expansion and differentiation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate. In an embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in culture. In another embodiment, the stem cells or progenitor cells are contacted with the compounds, composition, or conjugate in a subject. The compounds, composition or conjugate may be administered to a subject before,

during, or after implantation of stem cells in the subject to expand and differentiate the stem cells in the subject.

The invention also relates to a method for enhancing proliferation of insulin secreting cells in culture comprising contacting the cells with a GLP-1 agonist and a gastrin compound or a composition or conjugate of the invention in sufficient amounts to enhance proliferation of the cells. The amount of proliferation may be significantly different compared with that achieved in the absence of the compounds, composition or conjugate.

The invention also relates to a method for sustaining islet cells or precursor cells in culture comprising culturing the cells in the presence of a GLP-1 agonist and a gastrin compound or a composition or conjugate of the invention in an amount sufficient to sustain the cells in culture. The cells may be sustained in culture for a significantly longer period of time compared with cells cultured in the absence of the compounds, composition or conjugate. Culturing cells in the presence of a GLP-1 agonist and a gastrin compound or a composition or conjugate of the invention will be particularly useful in preparing and maintaining cells intended for transplantation.

The invention further relates to a method for treating a subject with a disease or condition described herein comprising contacting *ex vivo* a plurality of cells with a GLP-1 agonist and a gastrin compound, or a composition or conjugate of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

The invention still further relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with a GLP-1 agonist and a gastrin compound, composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

The invention provides methods for treating diabetes mellitus in a patient in need thereof by administering a composition comprising a gastrin compound and a GLP-1 agonist in an amount sufficient to effect differentiation of the patient's pancreatic islet precursor cells to mature insulin-secreting cells and/or to stimulate insulin sysnthesis in existing islet cells. The composition can be administered systemically or expressed *in situ* by host cells containing a nucleic acid construct in an expression vector wherein the nucleic acid construct comprises a coding sequence for a gastrin compound or a coding

sequence for a GLP-1 agonist, together with transcriptional and translational regulatory regions functional in pancreatic islet precursor cells.

Also provided are methods and compositions for treating diabetes in a patient in need thereof by implanting into a diabetic patient pancreatic islet cells that have been exposed in culture to a sufficient amount of a gastrin compound and a GLP-1 agonist to increase the number of pancreatic beta cells in the islets; optionally the population of pancreatic beta cells can be grown in culture for a time sufficient to expand the population of β-cells prior to transplantation.

Since the present invention relates to a method of treatment comprising a combination of active agents which may be administered separately or as conjugates, the invention also provides a kit comprising a GLP-1 agonist and a gastrin compound, a pharmaceutical composition, or conjugate of the invention in kit form.

The invention also contemplates the use of a composition comprising a combination of at least one GLP-1 agonist and at least one gastrin compound for the preparation of a medicament for preventing or treating a condition or disease. In an embodiment, the invention relates to the use of synergistically effective amounts of at least one GLP-1 agonist, and at least one gastrin compound for the preparation of a medicament for preventing or treating a condition or disease. The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for the prevention and/or treatment of diseases and conditions. The medicaments provide beneficial effects, preferably sustained beneficial effects following treatment.

These and other aspects, features, and advantages of the present invention should be apparent to those skilled in the art from the following detailed description.

**DEFINITIONS**

The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about." Further, it is to be understood that "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to a composition containing "a compound" includes a mixture of two or more compounds.

Selected compounds described herein contain one or more asymmetric centers and may give rise to enantiomers, diasteriomers, and other stereoisomeric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-. Therefore, the invention includes all such possible diasteriomers and enantiomers as well as their racemic and optically pure forms. Optically active (R)- and (S)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and A geometric isomers. All tautomeric forms are intended to be included within the scope of the invention.

The terms "subject", "individual" or "patient" refer to an animal including a warm-blooded animal such as a mammal, which is afflicted with or suspected of having or being pre-disposed to a disease or a condition as described herein. Preferably, the terms refer to a human. The terms also include domestic animals bred for food or as pets, including horses, cows, sheep, poultry, fish, pigs, cats, dogs, and zoo animals. The methods herein for use on subjects/individuals/patients contemplate prophylactic as well as curative use. Typical subjects for treatment include persons susceptible to, suffering from or that have suffered a condition or disease described herein.

The term "pharmaceutically acceptable carrier, excipient, or vehicle" refers to a medium which does not interfere with the effectiveness or activity of an active ingredient and which is not toxic to the hosts to which it is administered.

"Pharmaceutically acceptable salt(s)," includes salts of acidic or basic groups which may be present in the compounds suitable for use in the present invention. Examples of pharmaceutically acceptable salts include sodium, calcium and potassium salts of carboxylic acid groups and hydrochloride salts of amino groups. Other pharmaceutically acceptable salts of amino groups are hydrobromide, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, acetate, succinate, citrate, tartrate, lactate, mandelate, methanesulfonate (mesylate) and p-toluenesulfonate (tosylate) salts.

The term "about" means plus or minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made.

The term "preventing or treating" refers to the administration to a subject of biologically active agents either before or after onset of a condition or disease. A treatment may be either performed in an acute or chronic way.

A "beneficial effect" refers to an effect of a combination of a GLP-1 agonist and a gastrin compound, or composition or conjugate thereof, that is greater than the effect of either of the compounds alone. The beneficial effect includes favorable pharmacological and/or therapeutic effects, and improved pharmacokinetic properties and biological activity. A beneficial effect may be an additive effect or synergistic effect. In preferred embodiments of the invention, beneficial effects include but are not limited to the following: reduced or absent islet inflammation, decreased disease progression, increased survival, or cure of a disease or condition. In a particularly preferred embodiment, the beneficial effect is a "sustained beneficial effect" where the beneficial effect is sustained for a prolonged period of time after termination of treatment. In an embodiment, one or more of the aforementioned effects are sustained for a prolonged period of time after termination of treatment. A beneficial effect may be sustained for at least about 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment. The period of time a beneficial effect is sustained may correlate with the duration and timing of the treatment. A subject may be treated continuously for about 2 to 8 weeks, 2 to 12 weeks, 2 to 16 weeks, 2 weeks to 6 months, 2 weeks to 12 months, or periodically. A sustained beneficial effect may manifest as one or more of increased C-peptide production, increased pancreatic insulin production, and about normal or low blood glucose levels for a prolonged period following treatment.

The beneficial effect may be a statistically significant effect in terms of statistical analysis of an effect of the two compounds versus the effects of each of the compounds. "Statistically significant" or "significantly different" effects or levels with two compounds compared with each compound alone may represent levels that are higher or lower than a standard. In embodiments of the invention, the difference may be 1.5, 2, 3, 4, 5, or 6 times higher or lower compared with the effect obtained with each compound alone.

An "additive effect" of a GLP-1 agonist and a gastrin compound refers to an effect that is equal to the sum of the effects of the two individual compounds

A "synergistic effect" of a GLP-1 agonist and a gastrin compound refers to an effect that is greater than the additive effect which results from the sum of the effects of the two individual compounds.

A "combination treatment" or "administering in combination" means that the active ingredients are administered concurrently to a patient being treated. When administered in combination each component may be administered at the same time, or sequentially in any order at different points in time. Therefore, each component may be administered separately, but sufficiently close in time to provide the desired effect, in particular a beneficial, additive, or synergistic effect. The first compound may be administered in a regimen which additionally comprises treatment with the second compound.

"Therapeutically effective amount" relates to the amount or dose of active compounds (e.g. GLP-1 agonist and gastrin compound) or compositions or conjugates of the invention that will lead to one or more desired beneficial effects, preferably one or more sustained beneficial effects.

"Suboptimal dose" or suboptimal dosage" refers to a dose or dosage of an active compound which is less than the optimal dose or dosage for that compound when used in monotherapy.

An "analog" refers to a polypeptide wherein one or more amino acid residues of a parent polypeptide have been substituted by another amino acid residue, one or more amino acid residues of a parent polypeptide have been inverted, one or more amino acid residues of the parent polypeptide have been deleted, and/or one or more amino acid residues have been added to the parent peptide. Such an addition may be at either of the N-terminal or C-terminal end or within the parent polypeptide, or a combination thereof.

A "derivative" refers to a polypeptide in which one or more of the amino acid residues of a parent polypeptide have been chemically modified. A chemical modification includes adding chemical moieties, creating new bonds, and removing chemical moieties. A polypeptide may be chemically modified, for example, by alkylation, acylation, glycosylation, pegylation, ester formation, deamidation, or amide formation.

In the present context, "a GLP-1 agonist" is understood to refer to any compound, including peptides and non-peptide compounds, which fully or partially activate the human GLP-1 receptor. In a preferred embodiment, the "GLP-1 agonist" is any peptide or

non-peptide small molecule that binds to a GLP-1 receptor, preferably with an affinity constant (KD) or a potency ($EC_{50}$) of below 1 μM, for example, below 100 nM as measured by methods known in the art (see e.g. WO 98/08871) and exhibits insulinotropic activity, where insulinotropic activity may be measured *in vivo* or *in vitro* assays known to those of ordinary skill in the art.

A "GLP-1 agonist" includes naturally occurring or synthetic GLP-1 polypeptides, fragments, analogs, derivatives, and pharmaceutically acceptable salts thereof, and active metabolites and prodrugs of GLP-1. U.S. Patent No. 5,118,666 discloses GLP-1 agonists such as GLP-1(7-34) and GLP-1(7-35).

In reference to the nomenclature of analogs and derivatives, for example, $Gly^8$-GLP-1(7-37) designates a fragment of GLP-1 derived from GLP-1(7-37) by deleting the amino acid residues at positions 1 to 6, and substituting the amino acid residue in position 8 (Ala) by Gly.

Examples of analogs and derivatives of GLP-1 (including exendin-3 and exendin 4 analogs and derivatives) which can be used according to the present invention include those listed in Table 1 and also in WO 98/08871, WO 99/43705 (Novo Nordisk), WO 99/43706 (Novo Nordisk), WO 99/43707 (Novo Nordisk), WO 99/43708 (Novo Nordisk), WO 99/43341 (Novo Nordisk), PCT/DK99/00081 (Novo Nordisk A/S), PCT/DK99/00082 (Novo Nordisk A/S), PCT/DK99/0085 (Novo Nordisk A/S), WO 98/008871 (Novo Dordisk A/S), WO 87/06941 (The General Hospital Corporation), WO 90/11296 (The General Hospital Corporation), WO 91/11457 (Buckley et al), WO98/43658 (Eli Lilly & Co), EP 0708179-A2 (Eli Lilly & Co), WO 03/035099 (Eli Lilly & Co), WO 01/98331 (Eli Lilly & Co), EP 0699686-A2 (Eli Lilly & Co), US 2003/0224983 (Novo Nordisk A/S), , US 6268343 (Novo Nordisk A/S), US 200302249883-A1 (Novo Nordisk A/S), US 20030119734 A1 (Novo Nordisk A/S), US20040018975A1 (Eli Lilly & Co.), US 20030083259 A1 (Novo Nordisk A/S), US 20010006943-A1 (Novo Nordisk A/S), PCT/CA03/ 33595 (Transition Therapeutics), WO 99/43708, WO 00/66629, WO 01/04146, US Patent Nos. 5,977,071, 5,545,618, 5,705,483, 5,977,071, and 6,133,235; Adelhorst et al, J.Biol Chem. 1994, 269:6275; and Xiao, Q. et al, 2001, Biochemistry 40:2860-2869, which are incorporated herein by reference.

A "gastrin compound" includes a modified form of a gastrin such gastrin including but not limited to gastrin 34 (big gastrin), gastrin 17 (little gastrin), and gastrin 8 (mini

gastrin), pentagastrin, and tetragastrin. Sequences for gastrins including big gastrin-34 (Bonato et al , 1986, Life Science 39:959) and small gastrin-17 (Bentley et al (1966) Nature 209:583) are shown in SEQ ID NOs. 5-9. Modified gastrin compounds for use in the present invention comprise the modified gastrin compounds described in PCT/CA03/01778, US Serial No. 10/719,450 and U.S. Application Serial No. 60/519,933 incorporated in their entirety by reference. A gastrin compound may be selected that has a suitable $IC_{50}$, for example an $IC_{50}$ of about ~ 0.7 nM at a gastrin/CKK receptor, as measured by methods known in the art (see Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023 describing in vitro cell growth assays, and receptor binding assays as described in Singh et al (1995) J. Biol. Chem. 270: 8429-8438, and Kopin et al (1995) J. Biol. Chem. 270: 5019-5023).

A group of modified gastrin compounds include compounds having an amino acid sequence comprising from the amino terminus $Z\text{-}Y_m\text{-}X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$, wherein $AA_1$ is Tyr or Phe, $AA_2$ is Gly, Ala, or Ser, $AA_3$ is Trp, Val, or Ile, $AA_4$ is Met or Leu, $AA_5$ is Asp or Glu, and $AA_6$ is Phe or Tyr; Z is the sequence of a polymer or a protein (e.g. a serum protein such as human serum albumin); $Y_m$ is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 of SEQ ID NO: 5, providing that the gastrin compound binds a gastrin/CCK receptor. Generally, m is 0 to about 20 residues.

In preferred embodiments, X is one or more amino acid residues from position 28 to position 18 of SEQ ID NO: 5. Therefore, the gastrin compounds by virtue of the presence of X, can have any of gastrin sequences from positions 18-28, 19-28, 20-28, 21-28, etc. The gastrin compound optionally contains an amino acid spacer of length m, and m is 0 to about 20 residues.

A modified gastrin compound of the formula $X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$ where there is no spacer and m is 0, may further comprise a bifunctional cross-linking agent for linkage to Z, where Z further comprises a non-proteinaceous polymer.

A modified gastrin compound may further comprise an amino terminal cysteine or lysine residue.

In some embodiments of modified gastrin compounds described herein, the gastrin component contains at least amino acid residues 29-34 of SEQ ID NO: 5 or 6, and it is

associated with a polymer, a lipid or a carbohydrate. The polymer may be a synthetic or naturally occurring polymer. The term polymer includes a protein polymer of amino acids, and is not limited to a synthetic polymer. The polymer may be a polyethylene glycol (PEG) or a dextran. A modified gastrin compound can be based on SEQ ID NO: 5 or 5 or "big" gastrin-34 and have a residue at position 32 which is a methionine or a leucine, respectively.

Another preferred modified gastrin compound comprises a structure $Z\text{-}Y_m\text{-}X$, wherein Z is Cys or Lys, $Y_m$ is an optional spacer region comprising m amino acid residues of a small neutral amino acid, and X is at least six amino acid residues comprising at least positions 12-17 of gastrin-17 (SEQ ID NO: 7 or 8) or at least positions 29-34 of gastrin-34 (SEQ ID NO: 5 or 6). This modified gastrin compound can further comprise a bifunctional cross-linking agent wherein one reactive portion of the cross-linking agent is covalently linked to Z, and the other reactive portion is covalently linked to a polymer or protein.

In a preferred modified gastrin compound $AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$ is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe.

"Condition(s)" and/or "disease(s)" refer to one or more pathological symptoms or syndromes for which either or both a GLP-1 agonist or a gastrin compound provide a therapeutic effect. The condition or disease may require reduction of blood glucose levels, inhibition of gastric acid secretion, inhibition of apoptosis of β-cells, stimulation of proliferation or differentiation of β-cells, and reduction of body weight. Examples of conditions and diseases include but are not limited to dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, chronic heart failure, fluid retentive states, metabolic syndrome and related diseases and disorders, obesity, diabetic complications as well as symptoms of other diseases in which tissue is damaged due to elevated glucose levels, including Alzheimer's Disease, Parkinson's Disease, and other age-related, tissue-degenerative diseases, as well as the artherogenic effects of elevated leptin, for example in patients with impaired glucose tolerance and obese non-diabetic patients.

The term, "diabetes" as used herein means any manifested symptoms of diabetes in any mammal including experimental animal models, and including human forms such as type I and type II diabetes, early stage diabetes, and a pre-diabetic condition characterized by mildly decreased insulin or mildly elevated blood glucose levels. A "pre-diabetic condition" describes a subject demonstrating a symptom in terms of insulin or glucose level, and/or demonstrating a susceptibilty to diabetes or a related condition due to family history, genetic predisposition, or obesity in the case of type II diabetes, and includes a subject who has previously had diabetes or a related condition and is subject to risk of recurrence.

"Insulinotropic activity" refers to an ability of a substance to stimulate insulin secretion in response to elevated glucose levels to produce or increase glucose uptake by cells and decreased serum glucose or blood glucose levels. Methods known in the art can be employed to assay for insulinotropic activity. For example, *in vitro* and *in vivo* methods may be used that measures GLP-1 receptor binding activity or gastrin receptor binding activity, receptor activation (see the methods described in EP 619,322 to Gelfand et al and US Patent No. 5,120,712), insulin or C-peptide levels. Compounds, compositions or conjugates described herein have insulinotropic activity if islet cells secrete insulin in the presence of the compounds, compositions, or conjugates above background levels or levels in the absence of the compounds, compositions, or conjugates.

"Islet neogenesis" means formation of new beta cells by differentiation, which may or may not have the characteristics of stem cells which have the ability to reproduce in an unlimited manner.

## DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The invention is related to compositions, conjugates, and methods that utilize a GLP-1 agonist and a gastrin compound to provide beneficial effects.

The compositions, conjugates and methods of the invention provide enhanced beneficial effects, in particular sustained beneficial effects relative to a GLP-1 agonist and/or a gastrin compound alone. In embodiments of the invention, the beneficial effects are additive or synergistic.

In an embodiment, where the disease or condition is diabetes, sustained beneficial effects of a composition, combination treatment, or conjugate of the invention may manifest as one or more of the following:

- An increase in pancreatic insulin levels relative to the levels measured in the absence of the active compounds or for each compound alone after administration to a subject with symptoms of diabetes. Preferably the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in pancreatic insulin levels in a subject.
- A reduction of an absence of symptoms of islet inflammation after administration to a subject with symptoms of diabetes.
- A decrease in blood glucose levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds induce at least about a 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels. Most preferably, the compounds yield blood glucose levels about or close to the levels common in a normal subject.
- An increase in C-peptide levels relative to the levels measured in the absence of the compounds or for each compound alone in subjects with symptoms of diabetes. Preferably, the compounds together induce at least about a 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%, 15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in C-peptide levels.
- Maintenance of blood glucose levels at about normal for a prolonged period of time.
- A reduction, prevention, or slowing of the rate of disease progression in a subject with diabetes.
- A reduction or prevention of the development of severe hyperglycemia and ketoacidosis with symptoms of diabetes.
- An increase in survival in a subject with symptoms of diabetes.

One or more of these beneficial effects can be demonstrated in a diabetic subject or disease model, for example a non-obese (NOD) mouse with symptoms of diabetes.

A gastrin compound is selected for particular embodiments in the present invention and to provide a specific beneficial effect(s) based on characteristics including its insulinotrophic activity, the ability to augment the activity of a GLP-1 agonist (in

particular to enhance the insulinotropic effects of a GLP-1 agonist), and/or increase the physical or chemical stability of a GLP-1 agonist. A gastrin compound can also be selected based on its ability to stimulate proliferation/differentiation of beta cells, and its *in vivo* half-life.

In an embodiment of the invention, the gastrin compound is gastrin 17 and analogs and derivatives thereof. In a particular embodiment, the gastrin compound is synthetic human gastrin I having 17 amino acid residues with a Leu residue at amino acid position 15.

Gastrin compounds may be synthesized by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield, 1964, J. Am. Chem. Assoc. 85:2149-2154) or synthesis in homogenous solution (Houbenweyl, 1987, Methods of Organic Chemistry, ed. E. Wansch, Vol. 15 I and II, Thieme, Stuttgart). The synthesis may be performed using manual procedures or by automation. Automated synthesis may be carried out, for example, using an Applied Biosystems 431A peptide synthesizer (Perkin Elmer). Gastrin compounds may also be obtained from commercial sources. For example, synthetic human gastrin 17 with methionine or leucine at position 15 are available from Bachem AG, Bubendorf, (Switzerland), and from Research Plus Inc (New Jersey, USA).

A GLP-1 agonist may be selected for particular applications in the present invention based on one or more of the following characteristics: ability to bind to the GLP-1 receptor, preferably with an affinity constant $K_d$ less than about 1 μM, more preferably less than about 100nM; ability to initiate a signal transduction pathway resulting in insulinotropic activity; insulinotropic activity; stimulation of beta cell proliferation/differentiation; resistance to DP IV cleavage; and, an *in vivo* half-life of at least about 15 minutes to 24 hours, preferably 2 to 10 hours or 2 to 8 hours in humans using conventional methods (see for example, the method described in US 2003/0144206)

An amino acid portion of a GLP-1 agonist can be prepared by a variety of methods known in the art such as solid-phase synthesis, purification of GLP-1 agonists from natural sources, recombinant technology, or a combination of these methods. See for example, United States Patent Nos. 5,188,666, 5,120,712, 5,523,549, 5,512,549, 5,977,071, 6,191,102, Dugas and Penney 1981, Merrifield, 1962, Stewart and Young 1969, and the references cited herein.

In an embodiment of the invention the GLP-1 agonist is a naturally truncated GIP-1 polypeptide ((GLP-1(7-37) and GLP-1(7-36)amide) [SEQ ID NO. 10 and 2, respectively], or an analog or derivative thereof. The sequences of these naturally occurring truncated GLP-1 agonists are represented in SEQ ID NOs. 1 and 2. In aspects of the invention, a GLP-1 agonist may have the amino acid sequence of SEQ ID NOs. 1 or 2 modified so that amino acid residues at positions 1-20, preferably 1-15, more preferably 1-10, most preferably 1-5 differ from the sequences of SEQ ID NOs. 1 or 2.

In another embodiment of the invention, the GLP-1 agonist is an analog of GLP-1(7-37) or GLP-1(7-36) which has less than 10 amino acid residues that are different from those in GLP-1(7-37) or GLP-1(7-36), less than 5 amino acid residues that are different from those in GLP-1(7-37) or GLP-1(7-36), less than 3 amino acid residues that are different from those in GLP-1 (7-37) or GLP-1(7-36), preferably only one amino acid residue that is different from sequence of GLP-1(7-37) or GLP-1(7-36).

GLP-1 agonists that may have specific utility in the present invention include polypeptides where one or more amino acids have been added to the N-terminus and/or C-terminus of GLP-1(7-37) or GLP-1(7-36). Preferably, about one to six amino acids may be added to the N-terminus and/or from about one to eight amino acids may be added to the C-terminus. In certain applications GLP-1 agonists are selected that have up to 39 amino acids. Amino acids at positions 1-6 of an extended GLP-1 agonist may be selected so that they are the same or are conservative substitutions of the amino acid at the corresponding positions of the parent GLP-1(7-37) or GLP-1(7-36). Amino acids at positions 38-45 of an extended GLP-1 agonist may be selected so that they are the same or conservative substitutions of the amino acids at the corresponding positions of exendin-3 or exendin-4 (SEQ ID NO. 3 and 4 respectively).

In aspects of the invention a GLP-1 agonist is utilized comprising a position 8 analog wherein the backbone for such analogs or fragments thereof contain an amino acid other than alanine. The amino acid at position 8 may be selected from glycine, valine, leucine, isoleucine, serine, threonine, or methionine

In an embodiment, GLP-1 agonists are selected that have the sequence GLP-1(7-37)OH and GLP-1(7-36) amide, and the corresponding position 8 analogs wherein the backbone for such analogs contains an amino acid other than alanine. The amino acid at position 8 may be selected from glycine, valine, leucine, isoleucine, serine, threonine, or

methionine, preferably valine or glycine. The analogs may additionally contain (a) an amino acid at position 22 selected from glutamic acid, lysine, aspartic acid, arginine, and preferably glutamic acid or lysine; (b) an amino acid at position 30 selected from glutamic acid, aspartic acid, serine, or histidine; (c) an amino acid at position 37 selected from lysine, arginine, threonine, glutamic acid, aspartic acid, serine, tryptophan, tyrosine, phenylalanine, or histidine; and/or (d) amino acid at position 27 selected from alanine, lysine, arginine, tryptophan, tyrosine, phenylalanine, or histidine.

A group of GLP-1 analogs and derivatives for use in the present invention comprises the GLP-1 agonists described in U.S. Pat. No. 5,545,618 and US Patent Application Serial No. 20040018975. The analogs include active GLP-1 peptides, 7-34, 7-35, 7-36 and 7-37 having amino acid substitutions as positions 7-10 and/or are truncations at the C-terminus and/or contain various other amino acid substitutions in the basic peptide. Preferred analogs include those with D-amino acid substitutions in the 7 and 8 positions and/or N-alkylated or N-acylated amino acids in the 7 position since they are particularly resistant to degradation *in vivo*.

In another embodiment of the invention at least one amino acid of a GLP-1 agonist has at least one substituent attached directly or indirectly (e.g. via a spacer such as γ-Glu or β-Ala). A substituent is generally selected to make the profile of action of the parent GLP-1 agonist more protracted, make the GLP-1 agonists more metabolically and physically stable, and/or increase solubility of the GLP-1 agonist. An example of a particular substituent is a lipophilic substituent including but not limited to an alkyl group, a group which has an ω-carboxylic acid group, an acyl group of a straight-chain or branched fatty acid or alkane such as tetradecanoyl, hexadecanoyl. Particular compositions, conjugates and treatments of the invention use GLP-1 agonists with lipophilic substitutents such as those described in W0 99/43341 (Novo Nordisk) and US 2003/0119734A1 (Novo Nordisk). In a particular embodiment, the GLP-1 agonist is $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-Glu}(N^{\alpha}\text{-hexadecanoyl})))$-GLP-1(7-37).

In embodiments of the invention, the GLP-1 agonist is selected from the group consisting of: $Gly^8$-GLP-1(7-36)-amide, $Gly^8$-GLP-1(7-37), $Val^8$-GLP-1(7-36)-amide, $Val^8$-GLP-1(7-37), $Val^8Asp^{22}$-GLP-1(7-36)-amide, $Val^8Asp^{22}$-GLP-1(7-37), $Val^8Glu^{22}$-GLP-1(7-36)-amide, $Val^8Glu^{22}$-GLP-1(7-37), $Val^8Lys^{22}$-GLP-1(7-36)-amide, $Val^8Lys^{22}$-GLP-1(7-37) -$Val^8Arg^{22}$-GLP-1(7-36)-amide,

$Val^{8}Arg^{22}$-GLP-1(7-37), $Val^{8}His^{22}$-GLP-1(7-36)-amide, -$Val^{8}His^{22}$-GLP-1(7-37), $Arg^{26}$-GLP-1(7-37); $Arg^{34}$-GLP-1(7-37); $Lys^{36}$-GLP-1(7-37); $Arg^{26,34}Lys^{36}$-GLP-1(7-37); $Arg^{26,34}$-GLP-1(7-37); $Arg^{26,34}Lys^{40}$-GLP-1(7-37); $Arg^{26}Lys^{36}$-GLP-1(7-37); $Arg^{34}Lys^{36}$-GLP-1(7-37); $Val^{8}Arg^{22}$-GLP-1(7-37); $Met^{8}Arg^{22}$-GLP-1(7-37);$Gly^{8}His^{22}$-GLP-1(7-37); $Val^{8}His^{22}$-GLP-1(7-37); $Met^{8}His^{22}$-GLP-1(7-37);$His^{37}$-GLP-1(7-37); $Gly^{8}$-GLP-1(7-37); $Val^{8}$-GLP-1(7-37); $Met^{8}$-GLP-1(7-37);$Gly^{8}Asp^{22}$-GLP-1(7-37); $Val^{8}Asp^{22}$-GLP-1(7-37); $Met^{8}Asp^{22}$-GLP-1(7-37); $Gly^{8}Glu^{22}$-GLP-1(7-37); $Val^{8}$-GLP-1(7-37); $Gly^{8}Lys^{22}$-GLP-1(7-37); $Val^{8}Lys^{22}$-GLP-1(7-37); $Glu^{22}Met^{8}Glu^{22}$-GLP-1(7-37); $Gly^{8}Lys^{22}$-GLP-1(7-37); $Val^{8}Lys^{22}$-GLP-1(7-37); $Met^{6}Lys^{22}$-GLP-1(7-37); $Gly^{8}Arg^{22}$-GLP-1(7-37); $Val^{8}Lys^{22}His^{37}$-GLP-1(7-37); $Gly^{8}Glu^{22}His^{37}$-GLP-1(7-37); $Val^{8}Glu^{22}His^{37}$-GLP-1(7-37); $Met^{8}Glu^{22}His^{37}$-GLP-1(7-37);$Gly^{8}Lys^{22}$ $His^{37}$-GLP-1(7-37); $Met^{8}Lys^{22}His^{37}$-GLP-1(7-37);$Gly^{8}Arg^{22}His^{37}$-GLP-1(7-37); $Val^{8}Arg^{22}His^{37}$-GLP-1(7-37); $Met^{8}Arg^{22}His^{37}$-GLP-1(7-37); $Gly^{8}His^{22}His^{37}$-GLP-1(7-37); $Val^{8}His^{22}His^{37}$-GLP-1(7-37); $Met^{8}His^{22}His^{37}$-GLP-1(7-37); $Gly^{8}His^{37}$-GLP-1(7-37); $Val^{8}His^{37}$-GLP-1(7-37); $Met^{8}His^{37}$-GLP-1(7-37); $Gly^{8}Asp^{22}His^{37}$-GLP-1(7-37); $Val^{8}Asp^{22}His^{37}$-GLP-1(7-37); $Met^{8}Asp^{22}His^{37}$-GLP-1(7-37); $Arg^{26}$-GLP-1(7-36)-amide; $Arg^{34}$-GLP-1(7-36)-amide; $Lys^{36}$-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{36}$-GLP-1-(7-36)-amide; $Arg^{26,34}$-GLP-1(7-36)-amide; $Arg^{26,34}Lys^{40}$-GLP-1(7-36)-amid- e; $Arg^{26}Lys^{36}$-GLP-1(7-36)-amide; $Arg^{34}Lys^{36}$-GLP-1(7-36)-amide; $Gly^{8}$-GLP-1(7-36)-amide; $Val^{8}$-GLP-1(7-36)-amide; $Met^{8}$-GLP-1(7-36)-amide;$Gly^{8}Asp^{22}$-GLP-1(7-36)-amide; $Gly^{8}Glu^{22}His^{37}$-GLP-1(7-36)-amide; $Val^{8}Asp^{22}$-GLP-1(7-36)-amide; $Met^{8}Asp^{22}$-GLP-1(7-36)-amide;$Gly^{8}Glu^{22}$-GLP-1(7-36)-amide; $Val^{8}Glu^{22}$-GLP-1(7-36)-amide; $Met^{8}Glu^{22}$-GLP-1(7-36)-amide; $Gly^{8}Lys^{22}$-GLP-1(7-36)-amide; $Val^{8}Lys^{22}$-GLP-1(7-36)-amide; $Met^{8}Lys^{22}$-GLP-1(7-36)-amide; $Gly^{8}His^{22}His^{37}$-GLP-1(7-36)-amide; $Gly^{8}Arg^{22}$-GLP-1(7-36)-amide; $Val^{8}Arg^{22}$-GLP-1(7-36)-amide; $Met^{8}Arg^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^{8}His^{22}$-GLP-1(7-36)-amide; $Val^{8}His^{22}$-GLP-1(7-36)-amide; $Met^{8}His^{22}$-GLP-1(7-36-amide;$His^{37}$-GLP-1(7-36)-amide; $Val^{8}Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Met^{8}Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Gly^{8}His^{37}$-GLP-1(7-

36)-amide; $Val^8His^{37}$-GLP-1(7-36)-amide; $Met^8His^{37}$-GLP-1(7-36)-amide;$Gly^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Asp^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Glu^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Glu^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^8Lys^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8Lys^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8Lys^{22}His^{37}$-GLP-1(7-36)-amide;$Gly^8Arg^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8His^{22}His^{37}$-GLP-1(7-36)-amide; $Met^8His^{22}His^{37}$-GLP-1(7-36)-amide; $Val^8$-GLP-1(7-37)OH, $Gly^8$-GLP-1(7-37)OH, $Glu^{22}$-GLP-1(7-37)OH, $Asp^{22}$-GLP-1(7-37)OH, $Arg^{22}$-GLP-1(7-37)OH, $Lys^{22}$-GLP-1(7-37)OH, $Cys^{22}$-GLP-1(7-37)OH, $Val^8$-$Glu^{22}$-GLP-1(7-37)OH, $Val^8$-$Asp^{22}$-GLP-1(7-37)OH, $Val^8$-$Arg^{22}$-GLP-1(7-37)OH, $Val^8$-$Lys^{22}$-GLP-1(7-37)OH, $Val^8$-$Cys^{22}$-GLP-1(7-37)OH, $Gly^8$-$Glu^{22}$-GLP-1(7-37)OH, $Gly^8$-$Asp^{22}$-GLP-1(7-37)OH, $Gly^8$-$Arg^{22}$-GLP-1(7-37)OH, $Gly^8$-$Lys^{22}$-GLP-1(7-37)OH, $Gly^8$-$Cya^{22}$-GLP-1(7-37)OH, $Glu^{22}$-GLP-1(7-36), $NH_2$, $ASP^{22}$-GLP-1(7-36)$NH_2$, Arg $^{22}$-GLP-1(7-36)$NH_2$, $Lys^{22}$-GLP-1(7-36)$NH_2$, $Cys^{22}$-GLP-1(7-36)$NH_2$, $Val^8$-$Glu^{22}$-GLP-1(7-36)$NH_2$, $Val^8$-$Asp^{22}$-GLP-1(7-36)$NH_2$, $Val^8$-$Arg^{22}$-GLP-1(7-36)$NH_2$, $Val^8$-$Lys^{22}$-GLP-1(7-36)$NH_2$, $Val^8$-$Cys^{22}$-GLP-1(7-36)$NH_2$, $Gly^8$-$Glu^{22}$-GLP-1(7-36)$NH_2$, $Gly^8$-$Asp^{22}$-GLP-1(7-36)$NH_2$, $Gly^8$-$Arg^{22}$-GLP-1(7-36)$NH_2$, $Gly^8$-$Lys^{22}$-GLP-1(7-36)$NH_2$, $Gly^8$-$Cys^{22}$-GLP-1(7-36)$NH_2$, $Lys^{23}$-GLP-1(7-37)OH, $Val^8$-$Lys^{23}$-GLP-1(7-37)OH, $Gly^8$-$Lys^{23}$-GLP-1(7-37)OH, $His^{24}$-GLP-1(7-37)OH, $Val^8$-$His^{24}$-GLP-1(7-37)OH, $Gly^8$-$His^{24}$-GLP-1(7-37)OH, $Lys^{24}$-GLP-1(7-37)OH, $Val^8$-$Lys^{24}$-GLP-1(7-37)OH, Gly8-Lys23-GLP-1(7-37)OH, $Glu^{30}$-GLP-1(7-37)OH, $Val^8$-$Glu^{30}$-GLP-1(7-37)OH, $Gly^8$-$Glu^{30}$-GLP-1(7-37)OH, $Asp^{30}$-GLP-1(7-37)OH, $Val^8$-$Asp^{30}$-GLP-1(7-37)OH, $Gly^8$-$Asp^{30}$-GLP-1(7-37)OH, $Gln^{30}$-GLP-1(7-37)OH, $Val^8$-$Gln^{30}$-GLP-1(7-37)OH, $Gly^8$-$Gln^{30}$GLP-1(7-37)OH, $Tyr^{30}$-GLP-1(7-37)OH, $Val^8$-$Tyr^{30}$-GLP-1(7-37)OH, $Gly^8$-$Tyr^{30}$-GLP-1(7-37)OH, $Ser^{30}$-GLP-1(7-37)OH, $Val^8$-$Ser^{30}$-GLP-1(7-37)OH, $Gly^8$-$Ser^{30}$-GLP-1(7-37)OH, $His^{30}$-GLP-1(7-37)OH, $Val^8$-$His^{30}$-GLP-1(7-37)OH, $Gly^8$-$His^{30}$-GLP-1(7-37)OH, $Glu^{34}$-GLP-1(7-37)OH, $Val^8$-$Glu^{34}$-GLP-1(7-37)OH, $Gly^8$-$Glu^{34}$-GLP-1(7-37)OH, $Ala^{34}$-GLP-1(7-37)OH, $Val^8$-$Ala^{34}$-GLP-1(7-37)OH, $Gly^8$-$Ala^{34}$-GLP-1(7-37)OH, $Gly^{34}$-GLP-1(7-37)OH, $Val^8$-$Gly^{34}$-GLP-1(7-37)OH, $Gly^8$-$Gly^{34}$-GLP-1(7-37)OH, $Ala^{35}$-GLP-1(7-37)OH, $Val^8$-$Ala^{35}$-GLP-1(7-37)OH, $Gly^8$-$Ala^{35}$-GLP-1(7-37)OH, $Lys^{35}$-GLP-1(7-37)OH, $Val^8$-$Lys^{35}$-GLP-1(7-37)OH, $Gly^8$-$Lys^{35}$-GLP-1(7-37)OH, $His^{35}$-GLP-1(7-37)OH $Val^8$-$His^{35}$-GLP-1(7-37)OH, $Gly^8$-$His^{35}$-GLP-1(7-37)OH, $Pro^{35}$-GLP-1(7-37)OH, $Val^8$-$Pro^{35}$-GLP-1(7-37)OH, $Gly^8$-$Pro^{35}$-GLP-1(7-37)OH, $Glu^{35}$-GLP-1(7-37)OH, $Val^8$-

$Glu^{35}$-GLP-1(7-37)OH, $Gly^{8}$-$Glu^{35}$-GLP-1(7-37)OH, $Val^{8}$-$Ala^{27}$-GLP-1(7-37)OH, $Val^{8}$-$His^{37}$-GLP-1(7-37)OH, $Val^{8}$-$Glu^{22}$-$Lys^{23}$-GLP-1(7-37)OH, $Val^{8}$-$Glu^{22}$-$Glu^{23}$-GLP-1(7-37)OH, $Val^{8}$-$Glu^{22}$-$Ala^{27}$-GLP-1(7-37)OH, $Val^{8}$-$Gly^{34}$-$Lys^{35}$-GLP-1(7-37)OH, $Val^{8}$-$His^{37}$-GLP-1(7-37)OH, and $Gly^{8}$-$His^{37}$-GLP-1(7-37)OH, and derivatives thereof.and derivatives thereof.

In a particular embodiment the GLP-1 agonists are $Val^{8}$-GLP-1(7-37)OH, $Gly^{8}$-GLP-1(7-37)OH, $Glu^{22}$-GLP-1(7-37)OH, $Lys^{22}$-GLP-1(7-37)OH, $Val^{8}$-$Glu^{22}$-GLP-1(7-37)OH, $Val^{8}$- $Lys^{22}$-GLP-1(7-37)OH, $Gly^{8}$-$Glu^{22}$-GLP-1(7-37)OH, $Gly^{8}$-$Lys^{22}$-GLP-1(7-37)OH, $Glu^{22}$-GLP-1(7-36)$NH_2$, $Lys^{22}$-GLP-1(7-36)$NH_2$, $Val^{8}$-$Glu^{22}$-GLP-1(7-36)$NH_2$, $Val^{8}$-$Lys^{22}$-GLP-1(7-36)$NH_2$, $Gly^{8}$-$Glu^{22}$-GLP-1(7-36)$NH_2$, $Gly^{8}$-$Lys^{22}$-GLP-1(7-36)$NH_2$, $Val^{8}$-$His^{37}$-GLP-1(7-37)OH, $Gly^{8}$-$His^{37}$-GLP-1(7-37)OH, $Arg^{34}$-GLP-1(7-36)$NH_2$, and $Arg^{34}$-GLP-1(7-37)OH.

In another particular embodiment, the GLP-1 agonist is selected from the group consisting of $Gly^{8}$-GLP-1(7-37), $Val^{8}$GLP-1(7-37), $Val^{8}Asp^{22}$GLP-1(7-37), $Val^{8}Glu^{22}$GLP-1(7-37), $Val^{8}Lys^{22}$GLP-1(7-37), and $Val^{8}His^{22}$ GLP-1(7-37), and analogs and derivatives thereof.

In a further particular embodiment, the GLP-1 agonist is selected from the group consisting of $Gly^{8}$-GLP-1(7-36) amide, $Val^{8}$GLP-1(7-36) amide, $Val^{8}Asp^{22}$GLP-1(7-36) amide, $Val^{8}Glu^{22}$GLP-1(7-36) amide, $Val^{8}Lys^{22}$GLP-1(7-36) amide, and $Val^{8}His^{22}$ GLP-1(7-36) amide, and analogs and derivatives thereof.

Pharmaceutical compositions of the invention can be selected that have statistically significant beneficial effects, preferably sustained beneficial effects, compared with a GLP-1 agonist or a gastrin compound alone.

In an embodiment, a pharmaceutical composition with statistically significant beneficial effects, preferably sustained beneficial effects, is provided comprising a GLP-1 agonist selected from the group consisting of $Gly^{8}$-GLP-1(7-37), $Val^{8}$GLP-1(7-37), $Val^{8}Asp^{22}$GLP-1(7-37), $Val^{8}Glu^{22}$GLP-1(7-37), $Val^{8}Lys^{22}$GLP-1(7-37), $Val^{8}His^{22}$ GLP-1(7-37), $Arg^{34}Lys^{26}(N^{\varepsilon}(\gamma\text{-Glu}(N^{\alpha}\text{-hexadecanoyl})))$-GLP-1(7-37), $Gly^{8}$-GLP-1(7-36) amide, $Val^{8}$GLP-1(7-36) amide, $Val^{8}Asp^{22}$GLP-1(7-36) amide, $Val^{8}Glu^{22}$GLP-1(7-36) amide, $Val^{8}Lys^{22}$GLP-1(7-36) amide, and $Val^{8}His^{22}$ GLP-1(7-36) amide, and a gastrin compound having an amino acid sequence comprising, from the amino terminus, $Z\text{-}Y_m\text{-}X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$, wherein $AA_1$ is Tyr or Phe, $AA_2$ is Gly, Ala, or Ser,

$AA_3$ is Trp, Val, or Ile, $AA_4$ is Met or Leu, $AA_5$ is Asp or Glu, and $AA_6$ is Phe or Tyr; Z is the sequence of a polymer or a protein (e.g. a serum protein such as human serum albumin); $Y_m$ is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 of SEQ ID NO: 5, preferably $AA_1$-$AA_2$-$AA_3$-$AA_4$-$AA_5$-$AA_6$ is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe.

In an embodiment, the invention comprises pharmaceutically acceptable salts of a GLP-1 agonist and/or pharmaceutically acceptable salts of a gastrin compound.

In another embodiment, a pharmaceutical composition is provided which has been adapted for administration to a subject to provide sustained beneficial effects to treat a condition or disease, preferably diabetes. In a preferred embodiment, it is in a form such that administration to a subject results in blood glucose levels that are about normal that persist in the subject for a prolonged period of time after cessation of treatment.

In an embodiment, a composition or conjugate comprising a GLP-1 agonist and a gastrin compound have greater sustained insulinotropic activity following treatment compared with the activity of a GLP-1 agonist or gastrin compound alone or greater than GLP-1(7-37)OH.

This invention provides a conjugate comprising a GLP-1 agonist linked to a gastrin compound wherein the linkage is for example, via amino or carboxyl group. The invention also relates to isolated covalent conjugates of the invention, and compositions comprising covalent conjugates of the invention.

A GLP-1 agonist may be conjugated to a species via an ester bond between a OH and a COOH of a gastrin compound.

Conjugates of a GLP-1 agonist and a gastrin compound may be conjugated with an intermediate spacer or linker. A suitable spacer or linker may be a mono- or disaccharide, an amino acid, a sulfate, a succinate, an acetate, or an oligomeric polymeric spacer or linker comprising one or more of such moieties.

The invention also provides methods of preparing the above covalent conjugates that result in conjugates with improved pharmacokinetic properties, biological activity, and beneficial effects. The methods comprise incubating the GLP-1 agonist with the gastrin compound under conditions that allow formation of a covalent linkage between the two compounds.

The invention therefore contemplates a process for preparing a covalent conjugate comprising a GLP-1 agonist covalently bonded or linked to a gastrin compound, the process comprising: incubating the GLP-1 agonist with a gastrin compound under conditions and at a pH and for a time sufficient for formation of a covalent bond or linkage between the GLP-1 agonist and gastrin compound; and isolating the covalent conjugate.

The above process for preparing a conjugate comprising a GLP-1 agonist and a gastrin compound provides a conjugate with a substantial amount of a GLP-1 agonist covalently linked to the GLP-1 agonist.

N-terminal or C-terminal fusion proteins or chimeric proteins, comprising a GLP-1 agonist conjugated with a gastrin compound, optionally with a spacer or linker, may also be prepared by fusing, through recombinant techniques, the N-terminal or C-terminal sequence of a GLP-1 agonist and the sequence of a gastrin compound.

The invention also provides a conjugate prepared by a process described herein.

The invention also related to pharmaceutical formulations comprising conjugates of the invention and a pharmaceutically acceptable carrier, excipient, or vehicle.

The invention further relates to a pharmaceutical formulation of substantially pure covalent conjugates comprising a GLP-1 agonist covalently linked to a gastrin compound which provides beneficial effects preferably sustained beneficial effects compared to the GLP-1 agonist alone.

In an embodiment, a pharmaceutical formulation is provided consisting essentially of covalent conjugates comprising a GLP-1 agonist covalently linked without an intermediate spacer or linker to a gastrin compound.

According to another aspect of the invention, a kit is provided. The kit is a package which houses a container which contains a covalent conjugate of the invention and also houses instructions for administering the covalent conjugate to a subject.

In another aspect the invention provides a combination treatment for treating or preventing a condition or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one GLP-1 agonist and at least one gastrin compound to produce beneficial effects, preferably sustained beneficial effects.

The invention also relates to a method of treatment comprising administering a therapeutically effective amount of at least one GLP-1 agonist in combination with the administration of at least one gastrin compound which upon administration to a subject

with symptoms of diabetes produces beneficial effects, preferably sustained beneficial effects, manifested as reduced blood glucose levels and/or increased pancreatic insulin.

In an aspect of the invention therapeutically effective amounts of a GLP-1 agonist and a gastrin compound are combined prior to administration to a subject. In an embodiment, therapeutically effective amounts of a GLP-1 agonist and a gastrin compound are mixed at a physiologically acceptable pH.

The invention also contemplates the use of a composition or conjugate of the invention or combination treatment of the invention for preventing, and/or ameliorating disease severity, disease symptoms, and/or periodicity of recurrence of a disease or condition described herein. The invention also relates to the prevention and treatment, in mammals, of conditions or diseases using the compositions, combination treatments, and conjugates of the invention.

In an embodiment, the invention provides a method for stimulating beta cell proliferation in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a GLP-1 agonist and a gastrin compound.

In another embodiment, the invention provides a method for increasing the number and/or size of beta cells in a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention or administering in combination a GLP-1 agonist and a gastrin compound.

In a further embodiment, the invention provides a method for preventing or treating Type I or Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a GLP-1 agonist and a gastrin compound.

In a still further embodiment, the invention provides a method for amelioriating progression of disease or obtaining a less severe stage of disease in a person suffering from Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a GLP-1 agonist and a gastrin compound.

The invention relates to a method of delaying the progression of impaired glucose tolerance or non-insulin requiring Type II diabetes to insulin requiring Type II diabetes comprising administering a therapeutically effective amount of a composition or conjugate

of the invention, or administering in combination a GLP-1 agonist and a gastrin compound.

The invention also relates to a method of increasing the insulin synthesis capability of a subject comprising administering a therapeutically effective amount of a composition or conjugate of the invention, or administering in combination a GLP-1 agonist and a gastrin compound.

The invention provides methods for treating cells, preferably cells in culture using a GLP-1 agonist and gastrin compound of the invention, or compositions, or conjugates of the invention. The invention also provides cell based treatment methods using a GLP-1 agonist and gastrin compound of the invention, or compositions, or conjugates of the invention. See PCT/CA03/33595 for a description of general culture and cell based treatment methods.

In an aspect, the invention provides a method of treating a disease or condition comprising administering a GLP-1 agonist and a gastrin compound, a composition or conjugate of the invention with a plurality of cells to a subject in need thereof to thereby produce a beneficial effect, preferably a sustained beneficial effect.

A method for treating a subject with a disease or condition described herein comprising contacting *ex vivo* a plurality of cells with a GLP-1 agonist and a gastrin compound, or a composition or conjugate of the invention of the invention, optionally culturing the cells, and administering the cells to the subject in need thereof.

In embodiments of the aforementioned cell based therapeutic methods the cells are pancreatic ductal cells and the amount of compounds/composition/conjugate used in the method is generally effective to increase the amount of insulin secreting cells in the subject. The cells may be autologous (i.e. from the same subject), or may be from another individual of the same species, or from a different species.

The methods of the invention may further comprise measuring one or more of the following markers: blood glucose, serum glucose, blood glycosylated haemoglobin, pancreatic beta cell mass, serum insulin, pancreatic insulin levels, morphometrically determined beta cell mass, amount of insulin secreting cells, and glucose responsiveness of insulin secreting cells.

The invention also relates to inducing islet neogenesis in a subject comprising contacting islet precursor cells with a GLP-1 agonist and a gastrin compound,

composition, or conjugate of the invention in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

The invention also relates to a method for expanding and differentiating stem cells into insulin secreting cells comprising contacting the stem cells with an effective amount of a GLP-1 agonist and a gastrin compound or a composition or conjugate of the invention. The compounds, composition or conjugates may be administered to a subject before, during, or after stem cells are implanted in the subject. The stem cells may be obtained from pancreatic islets, umbilical cords, embryos, or stem cell lines. The method may additionally comprise administering an immunosuppressive agent.

The invention contemplates a method of expanding a functional beta cell mass of pancreatic islet transplants in a diabetic patient, the method comprising administering to the patient a therapeutically effective amount of a GLP-1 agonist and a gastrin compound, or a composition or conjugate of the invention.

The invention also contemplates a method for treating diabetes in a subject comprising transplanting a pancreatic islet preparation into the subject and administering a therapeutically effective amount of a GLP-1 agonist and a gastrin compound, or a composition or conjugate of the invention.

Since the present invention relates to a method of treatment comprising a combination of active agents which may be administered separately or as conjugates, the invention also provides a kit comprising a GLP-1 agonist and a gastrin compound, a pharmaceutical composition or conjugate in kit form. The invention also provides a pharmaceutical kit comprising one bottle with a GLP-1 agonist and another bottle with a gastrin bottle in one box.

The invention also contemplates the use of a composition comprising a combination of at least one GLP-1 agonist and at least one gastrin compound for the preparation of a medicament providing beneficial effects, preferably sustained beneficial effects in treating a condition or disease.

In an embodiment, the invention relates to the use of a therapeutically effective amount of at least one GLP-1 agonist, and at least one gastrin compound for preparation of a medicament for providing beneficial effects, preferably sustained beneficial effects, in treating a condition or disease.

In an embodiment the invention provides the use of a GLP-1 agonist and a gastrin compound for the preparation of a medicament for increase (preferably sustained increase) of the number and/or size of beta cells in a subject after treatment.

In another embodiment the invention provides the use of GLP-1 agonist and a gastrin compound for the preparation of a medicament for stimulation (preferably sustained stimulation) of beta cell proliferation after treatment.

In a still further embodiment the invention provides the use of GLP-1 and Gastrin for the preparation of a medicament for treatment of Type I or Type II diabetes.

The invention additionally provides uses of a pharmaceutical composition and a conjugate of the invention in the preparation of medicaments for beneficial effects, preferably sustained beneficial effects, in the treatment of diseases and conditions.

Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals such as by calculating a statistical parameter such as the $ED_{50}$ ( the dose that is therapeutically effective in 50% of the population) or $LD_{50}$ (the dose lethal to 50% of the population) statistics. The therapeutic index is the dose ratio of therapeutic to toxic effects and it can be expressed as the $ED_{50}/LD_{50}$ ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

The compositions of the present invention can be administered by any means that produce contact of the active agent(s) with the agent's sites of action in the body of a subject or patient. The active ingredients can be administered simultaneously or sequentially, and in any order at different points in time, to provide the desired beneficial effects. The compounds, conjugates and compositions can be formulated for sustained release, for delivery locally or systemically. It lies within the capability of a skilled physician or veterinarian to select a form and route of administration that optimizes the effects of the compositions, conjugates, and treatments of the present invention.

The compositions may be administered in oral dosage forms such as tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. They may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular forms, all utilizing dosage forms well known to those of ordinary skill in the pharmaceutical arts. The compositions of the invention may be administered by intranasal

route via topical use of suitable intranasal vehicles, or via a transdermal route, for example using conventional transdermal skin patches. A dosage protocol for administration using a transdermal delivery system may be continuous rather than intermittent throughout the dosage regimen.

A preferred route of administration is parenteral administration, more preferably peripheral parenteral administration. Parenteral administration is generally understood to refer to the injection of a dosage form into the body by a sterile syringe or some other mechanical device such as an infusion pump. For the purpose of the present invention parenteral routes include intravenous, intramuscular, subcutaneous, and intraperitoneal routes of administration. For parenteral administration, the compounds or conjugates described herein may be combined with distilled water at an appropriate pH.

The present invention includes combination treatments providing additive or synergistic activity, delivering an additive or synergistically effective amount, or an amount to provide a therapeutically effective amount of a GLP-1 agonist and a gastrin compound, or a conjugate or composition of the invention. Therefore, pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in a synergistically effective amount or a therapeutically effective amount.

The dosage regimen of the invention will vary depending upon known factors such as the pharmacodynamic characteristics of the agents and their mode and route of administration; the species, age, sex, health, medical condition, and weight of the patient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, the route of administration, the renal and hepatic function of the patient, and the desired effect. The effective amount of a drug required to prevent, counter, or arrest progression of a condition can be readily determined by an ordinarily skilled physician or veterinarian.

A composition or treatment of the invention may comprise a unit dosage of at least one GLP-1 agonist and a unit dosage of at least one gastrin compound. A "unit dosage" refers to a unitary i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically and chemically stable unit dose comprising either the active agents as such or a mixture with one or more solid or liquid pharmaceutical excipients, carriers, or vehicles.

In an aspect, a pharmaceutical composition is provided comprising a therapeutically effective suboptimal dosage of a GLP-1 agonist and a gastrin compound that are more effective at decreasing or reducing glucose levels for a sustained period following treatment compared with a dosage of either a gastrin compound or GLP-1 agonist alone.

In another aspect, an improved pharmaceutical composition is provided comprising therapeutically effective suboptimal amounts of a GLP-1 agonist and a gastrin compound in a form for chronic or acute therapy of a disease or condition, in particular diabetes.

In an embodiment, the composition comprises a GLP-1 agonist and a gastrin compound in doses that are equal to or at least 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound required to provide beneficial effects, preferably sustained beneficial effects, to treat a disease or condition.

In an aspect the invention provides a pharmaceutical composition comprising between 0.5 to 6000, 100-1500, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms GLP-1 agonist per single unit and 0.5 to 6000, 100-3000, 100-6000, 1000-6000, 2000-6000, and 3000-6000 micrograms gastrin compound per single unit..

In another aspect the invention provides a pharmaceutical composition comprising between 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day GLP-1 and 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day gastrin compound.

A composition or formulation of the invention may administered to a subject continuously for 2 weeks to 12 months, 2 weeks to 6 months, 2-16 weeks, 2 weeks to 12 weeks, and/or 2-8 weeks, or periodically.

In an embodiment, the ratio of GLP-1 agonist to gastrin compound in a composition of the invention is selected to augment the activity of the GLP-1 agonist and/or gastrin compound and to provide beneficial effects, preferably sustained beneficial effects.

A GLP-1 agonist and a gastrin compound may be in a ratio selected to augment the activity of one or both compounds to produce beneficial effects, preferably an additive or synergistic effect, or beneficial effects, preferably sustained beneficial effects. In particular embodiments, the ratio of a GLP-1 agonist to a gastrin compound may be from 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, 1:1 to 1:5, and 1:1. In

other particular embodiments, the ratio of a gastrin compound to a GLP-1 agonist may be from 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, and 1:1 to 1:5.

In an embodiment, a GLP-1 agonist may be used in combination with a gastrin compound at therapeutically effective weight ratios of between about 1:1 to 1:150, preferably 1:1 to 1:50. In another embodiment, a gastrin compound may be used in combination with a GLP-1 agonist at therapeutically effective weight ratios of between about 1:1 to 1:150, preferably 1:1 to 1:50.

The compositions of the present invention or fractions thereof typically comprise suitable pharmaceutical diluents, excipients, vehicles, or carriers selected based on the intended form of administration, and consistent with conventional pharmaceutical practices. The carriers, vehicles etc. may be adapted to provide an additive, synergistically effective or therapeutically effective amount of the active compounds.

Suitable pharmaceutical diluents, excipients, vehicles, and carriers are described in the standard text, Remington's Pharmaceutical Sciences, Mack Publishing Company. By way of example for oral administration in the form of a capsule or tablet, the active components can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, methyl cellulose, magnesium stearate; glucose, calcium, sulfate, dicalcium phosphate, mannitol, sorbital, and the like. For oral administration in a liquid form, the drug components may be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water, and the like. Suitable binders (e.g. gelatin, starch, corn sweeteners, natural sugars including glucose; natural and synthetic gums, and waxes), lubricants (e.g. sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, and sodium chloride), disintegrating agents (e.g. starch, methyl cellulose, agar, bentonite, and xanthan gum), flavoring agents, and coloring agents may also be combined in the compositions or components thereof.

In an aspect of the invention a pharmaceutical composition has a pH from about 7 to 10.

Formulations for parenteral administration of a composition of the invention may include aqueous solutions, syrups, aqueous or oil suspensions and emulsions with edible oil such as cottonseed oil, coconut oil or peanut oil. Dispersing or suspending agents that

can be used for aqueous suspensions include synthetic or natural gums, such as tragacanth, alginate, acacia, dextran, sodium carboxymethylcellulose, gelatin, methylcellulose, and polyvinylpyrrolidone.

Compositions for parenteral administration may include sterile aqueous or non-aqueous solvents, such as water, isotonic saline, isotonic glucose solution, buffer solution, or other solvents conveniently used for parenteral administration of therapeutically active agents. A composition intended for parenteral administration may also include conventional additives such as stabilizers, buffers, or preservatives, e.g. antioxidants such as methylhydroxybenzoate or similar additives.

In an embodiment, a solid form pharmaceutical composition is provided (e.g. tablets, capsules, powdered, or pulverized form) comprising a crystalline or amorphous GLP-1 agonist and a crystalline or amorphous gastrin compound.

In another embodiment, the invention relates to a liquid drug formulation comprising pharmaceutically acceptable salts of a GLP-1 agonist and a gastrin compound, and to lyophilized drug formulations that can be reconstituted to provide suspensions that are stable and suitable for parenteral administration.

In a particular embodiment, the invention relates to an aqueous composition comprising pharmaceutically acceptable salts of a GLP-1 agonist and a gastrin compound, and a solvent system which effects solubilization. The invention also provides a drug comprising an aqueous formulation of pharmaceutically acceptable salts of a GLP-1 agonist and a gastrin compound with at least one solubilizer.

A composition of the invention may be sterilized by, for example, filtration through a bacteria retaining filter, addition of sterilizing agents to the composition, irradiation of the composition, or heating the composition. Alternatively, the compounds, conjugates, and compositions of the present invention may be provided as sterile solid preparations e.g. lyophilized powder, which are readily dissolved in sterile solvent immediately prior to use.

In addition to the formulations described herein, the compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the fractions may be formulated with suitable polymeric or

hydrophobic materials (for example, as an emulsion in an acceptable oil), or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions of the invention and components thereof may comprise soluble polymers as targetable drug carriers.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labelled for treatment of an indicated condition. For administration of a composition of the invention, such labelling would include amount, frequency, and method of administration.

The present invention also includes methods of using the compositions of the invention in combination with one or more additional therapeutic agents including without limitation immunosuppressive agents, antiobesity agents, antidiabetic agents, appetite regulating drugs, antihypertensive agents, agents for the treatment and/or prevention of complications resulting from or associated with a condition or disease, in particular diabetes and obesity, anti-nausea, anti-headache medications, and general medications that treat or prevent side effects

The invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

**Example 1**

**Comparison of modified gastrin compounds /conjugates of PCT/CA03/01778 and unmodified gastrin in combination with GLP-1 in preventing diabetes progression in NOD mice with recent onset diabetes**

The effect of treatment by a combination of GLP-1 and unmodified gastrin and GLP-1 and modified gastrin compounds /conjugates will be examined in NOD mice with recent onset diabetes, to determine whether administration of both GLP-1 and gastrin prevents severe hyperglycemia as well as increase pancreatic insulin content in NOD mice with recent-onset diabetes. The GLP-1 to be used is the GLP-1 biologically active fragment of human/mouse GLP-1 (having residues at positions 7-36 compared to the precursor from which the fragment is processed; obtained from Bachem H6795). Gastrin compounds /conjugates to be used are as follows: Compound B - - gastrin as synthetic

human gastrin I having 17 amino acid residues with a Leu residue at amino acid position 15, Compound E – gastrin as synthetic human gastrin I having 2-17 amino acid residues, Compound Q gastrin as synthetic human gastrin I having 2-17 amino acid residues with a HAS polymer linked via $(GA)_5$.

Non-obese diabetic (NOD) female mice, ages 12-14 weeks, will be monitored for development of onset of diabetes (fasting blood glucose > 8.0 to 15 mmol/l), and within 48 hours after onset of symptoms, four groups of mice will each be treated as follows: one group will be treated with vehicle only; and the other group will be administered 100 µg/kg/day of GLP-1, and the remaining groups will be treated with a combination of GLP-1 (100 µg/kg/day) and gastrin compound (3 µg/kg/day gastrin equivalent), each treatment administered via the intraperitoneal route daily.

Therapy will be administered for 14 days to 18 days. Animals will be monitored weekly for fasting blood glucose (FBG) levels. FBG levels will be measured at about 12 hours after food has been withdrawn, and 24 hours after the last peptide or vehicle injection. Upon cessation of therapy, all mice will be monitored for FBG levels for the next 4 weeks (weeks 2-6) so as to determine whether prevention of hyperglycemia persisted after termination of therapeutic treatment. At 14 days to 18 days treatment will be stopped.

The protocol includes sampling of these mice for data again at 6 weeks, and blood collecting blood for assay of FBG and plasma C-peptide, and sacrificing the mice for pancreatic insulin determinations and scoring of islet inflammation (insulitis). From the outset of treatment, mice will neither receive insulin-replacement treatment nor immunosuppression. The following parameters will be assessed: survival rates, pancreatic insulin levels, presence of islet inflammation and fasting blood glucose levels.

The data will demonstrate that GLP-1 in combination with a modified gastrin compounds /conjugates (Compound E or Q) with longer half lives are more effective in reducing blood glucose levels in diabetic animals compared to GLP-1 with native gastrin (Compound B). The data will support the use of longer lasting modified gastrin compounds /conjugates with GLP-1.

**Example 2**

Using standard Fmoc synthesis, two different "reactive" gastrin compounds will be produced: Compound 1 is a Modified gastrin-17 peptide that has an additional cysteine at

the N-terminal end; Compound 2 is a Modified gastrin-17 peptide that has an additional 10 amino acids of alternating glycine and alanine (5 amino acids each) as a spacer region with an additional cysteine at the N-terminal end.

Non-obese diabetic (NOD) female mice will be monitored for diabetes development (determined to be a fasting blood glucose, FBG level of greater than 6.6 mmol/l), and upon onset of diabetes, will be divided into four groups. Mice will be treated with either vehicle as a control; or with gastrin-17, with Compound A, or with Compound B (same molar concentration of active ingredient, i.e. gastrin, is to be used for all three gastrin treated groups), administered via intraperitoneal injection (i.p.) once daily for 14 days.

Fasting blood glucose (FBG) levels and pancreatic insulin levels will be measured determined. In addition, the serum half-life of gastrin will be measured as well as the circulating serum levels of gastrin

It is anticipated that of the three gastrin-treated groups, both groups of NOD mice that are treated either with Compound A or Compound B will maintain higher circulating levels of serum gastrin. In addition, the half-life of gastrin measured will be longer in mice treated with either Compound A or B as compared to the unmodified gastrin.

In addition, it is also anticipated that as compared to the vehicle treated control group which records increasingly high FBG levels, all three treated groups of animals will have decreased FBG levels. However, animals treated with either Compound A or Compound B will have lower FBG levels as well as increased pancreatic insulin levels compared to animals treated with unmodified gastrin, indicating an increased efficacy with a more long-acting gastrin compound.

The present invention is not to be limited in scope by the specific embodiments described herein, since such embodiments are intended as but single illustrations of one aspect of the invention and any functionally equivalent embodiments are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All publications, patents and patent applications referred to herein are incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. The citation of any reference herein is not an admission that such reference is available as prior art to the instant invention.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise.

**Table 1**

| *GLP-1 agonist* | *Source* |
|---|---|
| DAC:GLP-1 | Conjuchem |
| Long-lasting synthetic glucagons-like peptide | Conjuchem |
| Long-lasting insulinotropic peptides | Conjuchem |
| AC2592 | Amylin Pharmaceuticals/ Restoragen |
| AC2993 – Exenatide | Amylin Pharmaceuticals |
| Exendin-4 | Eli Lilly, Alkermes, Amylin |
| NN2211 - GLP-1 (Liraglutide) | Novo Nordisk |
| ThGLP-1 | Theratechnologies |
| ZP10 | Zealand Pharma/ Aventis |
| Albumin:GLP-1 fusion peptide | Human Genome Sciences |
| BIM 51077 | Roche/Ipsen |
| N-terminally truncated GLP-1 derivatives & analogs (lipophilic substituent attached) | Novo Nordisk<br>PCT/DK99/00081 |
| Derivatives of GLP-1 analogs with a lipophilic substituent | Novo Nordisk<br>PCT/DK99/00082<br>US 6,458,924 |
| N-terminally modified GLP-1 derivatives & analogs with lipophilic substituent attached and protracted profile of action (N-terminal end has a substituent comprising an optionally substituted 5- or 6-membered ring system) | Novo Nordisk<br>PCT/DK99/00085 |
| Derivatives of GLP-1 analogs with a lipophilic substituent (protracted profile of action) | Novo Nordisk<br>WO 98/08871 |
| GLP-1 fragment as insulinotropic hormone | The General Hospital Corporation<br>WO 87/06941 |
| GLP-1 derivatives with insulinotropic activity | The General Hospital Corporation<br>WO 90/11296 |
| GLP-1 analogs exhibiting enhanced stability or an enhanced capacity to stimulate insulin production | Buckley et al.<br>WO 91/11457 |

| | |
|---|---|
| GLP-1 analogs and derivatives (stimulate the secretion or biosynthesis of insulin in poorly functioning beta cells) | Eli Lilly & Co.<br>EP 0708179-A2 |
| N-terminal truncated GLP-1 and analogs (promote glucose uptake by cells but do not stimulate insulin expression or secretion) | Eli Lilly & Co.<br>EP 0699686 -A2 |
| GLP-1 analogs or derivatives for increasing the number and/or the size of beta cells and for stimulating beta cell proliferation | Novo Nordisk<br>US 2003/0224983 |
| GLP-1 derivatives with a lipophilic substituent and protracted profile of action | Novo Nordisk<br>US 6268343 |
| Pharmaceutical formulations of GLP-1 agonists | Novo Nordisk<br>US 20030119734 A1 |
| GLP-1 amide, fragment, analogue or derivative | Novo Nordisk<br>US 20030083259 A1 |
| GLP-1 compositions having protracted action | Novo Nordisk<br>US 20010006943 A1 |
| GLP-1 & gastrin | Transition Therapeutics<br>PCT/CA03/ |
| Gastrin formulations | Transition Therapeutics<br>PCT/CA03/ |
| Derivatives of GLP-1 analogs with a lipophilic substituent (protracted profile of action) | Novo Nordisk<br>WO 99/43706 |
| GLP-1 and exendin derivatives with just one lipophilic substituent attached to the C-terminal amino acid residue | Novo Nordisk<br>WO 99/43708 |
| Modified exendins and agonists linked to one or more polyethylene glycol polymers | Amylin Pharmaceuticals<br>WO 00/66629 |
| Ecarin, a procoagulant protein from Echis carinatus venom | Cohesion Technologies<br>WO 01/04146 |
| Modified Fragments of GLP-1, exendin 3 and exendin 4 | Conjuchem, Inc.<br>US 6,514,500 |
| GLP-1 analogs | Novo Nordisk A/S<br>US 6,451,974 |
| GLP-1 analogs, derivatives and active peptides | Eli Lilly and Company<br>6,191,102 |

| | |
|---|---|
| GLP-1 Fragments | The General Hospital Corporation<br>6,162,907 |
| GLP-1 molecules associated with a divalent metal cation | Eli Lilly and Company<br>6,133,235<br>5,977,071 |
| Buccal delivery systems with GLP-1 | Theratech, Inc.<br>5,863,555 |
| GLP-1 Analogs | Eli Lilly and Company<br>5,981,488 |
| GLP-1 mimics | Bristol-Myers Squibb Company<br>WO 03/033671 |
| Long lasting GLP-1 | Conjuchem, Inc.<br>US 6,593,295<br>US 6,514,500<br>US 6,329,336 |
| Precursor GLP-1 | Genzyme Corporation<br>WO 03/014318 |
| GLP-1 complexes | Eli Lilly and Company<br>6,358,924 |
| Modified peptides | Theratechnologies Inc.<br>WO 02/10195 |
| GLP-1 and related molecules | Zealand Pharma A/S<br>WO 2004/005342 |

**WHAT IS CLAIMED IS:**

1. A pharmaceutical composition comprising a GLP-1 agonist and a gastrin compound that provides beneficial effects relative to each compound alone, and optionally a pharmaceutically acceptable carrier, excipient, or vehicle.
2. A pharmaceutical composition as claimed in claim 1 that provides sustained beneficial effects.
3. A pharmaceutical composition as claimed in claim 2 in a form that provides normal blood glucose levels in a subject that persist for a prolonged period of time after administration.
4. A pharmaceutical composition as claimed in any preceding claim comprising therapeutically effective amounts of a GLP-1 agonist and a gastrin compound in a form for chronic or acute therapy of a subject in need thereof.
5. A pharmaceutical composition as claimed in claim 4 wherein the therapeutically effective amounts are suboptimal relative to the amount of each compound administered alone for treatment of diabetes.
6. A pharmaceutical composition as claimed in any preceding claim wherein the ratio of GLP-1 agonist to gastrin compound is selected to augment the activity of the GLP-1 agonist or gastrin compound.
7. A pharmaceutical composition as claimed in claim 5 wherein the ratio of a GLP-1 agonist to a gastrin compound is from about 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, 1:1 to 1:5, and 1:1.
8. A pharmaceutical composition as claimed in claim 6 wherein the ratio of a gastrin compound to a GLP-1 agonist is from about 1:1 to 1:110, 1:1 to 1:100, 1:1 to 1:75, 1:1 to 1:50, 1:1 to 1:25, 1:1 to 1:10, and 1:1 to 1:5.
9. A pharmaceutical composition as claimed in any preceding claim wherein the GLP-1 agonist is used in combination with the gastrin

compound at therapeutically effective weight ratios of between about 1:1.5 to 1:150, preferably 1:2 to 1:50.

10. A pharmaceutical composition as claimed in any preceding claim wherein the GLP-1 agonist and the gastrin compound are present in doses that are at least about 1.1 to 1.4, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, or 10 fold lower than the doses of each compound alone required to treat a disease or condition.

11. A pharmaceutical composition as claimed in claim 1 comprising an additive amount or synergistically effective amount of the GLP-1 agonist and the gastrin compound in a pharmaceutically acceptable excipient, carrier, or vehicle.

12. A pharmaceutical composition as claimed in claim 1 comprising between 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day GLP-1 agonist and 0.1 to 20, 0.1 to 30, 0.1 to 40, 0.1 to 50, and 0.1 to 60 micrograms/kg/day gastrin compound.

13. A pharmaceutical composition as claimed in claim 2 wherein the beneficial effects are one or more of the following: reduced or absent islet inflammation, decreased disease progression, increased survival, or decreased symptoms of a disease or condition.

14. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effects are sustained beneficial effects that persist for a prolonged period of time after termination of treatment.

15. A pharmaceutical composition as claimed in claim 13 wherein the beneficial effects are sustained for at least about 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.

16. A pharmaceutical composition as claimed in claim 13 wherein the sustained beneficial effects may manifest as increased C-peptide production, increased pancreatic insulin production, and about normal or low blood glucose levels for a prolonged period following treatment.

17. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is at least about a 0.5%, 1%, 2%, 5%, 10%,

15%, 20%, 30%, 33%, 35%, 40%, 45%, or 50% increase in pancreatic insulin levels.

18. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is at least about a 2%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% decrease in blood glucose levels.

19. A pharmaceutical composition as claimed in any preceding claim wherein the beneficial effect is a decrease in blood glucose levels for a period of at least about 2 to 4 weeks, 2 to 8 weeks, 2 to 12 weeks, 2 to 24 weeks, 2 weeks to 12 months, and 2 weeks to 18 months following treatment.

20. A pharmaceutical composition as claimed in any preceding claim wherein the GLP-1 agonist is a GLP-1(1-37), GLP-1(7-36) amide, fragments, analogs, and derivatives thereof, and active metabolites and prodrugs of GLP-1.

21. A pharmaceutical composition as claimed in any preceding claim wherein the GLP-1 agonist comprises a parent polypeptide of the formula GLP-1(7-R) wherein R is 36, 37, 38, 39, 40, 41, 42, 43, 44, and 45, and wherein optionally up to 5, 10, or 15 amino acid residues are replaced with any α-amino acid residue.

22. A pharmaceutical composition as claimed in any preceding claim wherein the GLP-1 agonist is an analog or derivative of GLP-1 listed in Table 1.

23. A pharmaceutical composition as claimed in any preceding claim wherein the gastrin compound is a compound of the formula $Z\text{-}Y_m\text{-}X_n\text{-}AA_1\text{-}AA_2\text{-}AA_3\text{-}AA_4\text{-}AA_5\text{-}AA_6$, wherein $AA_1$ is Tyr or Phe, $AA_2$ is Gly, Ala, or Ser, $AA_3$ is Trp, Val, or Ile, $AA_4$ is Met or Leu, $AA_5$ is Asp or Glu, and $AA_6$ is Phe or Tyr; Z is the sequence of a polymer or a protein; $Y_m$ is an optional spacer region comprising m amino acid residues of a small neutral amino acid including but not limited to serine and alanine, and X is any consecutive portion of residues 1-28 of SEQ ID NO: 5,

preferably $AA_1$-$AA_2$-$AA_3$-$AA_4$-$AA_5$-$AA_6$ is Tyr-Gly-Trp-Met-Asp-Phe or Tyr-Gly-Trp-Leu-Asp-Phe.

24. A method for treating or preventing a condition or disease in a subject comprising administering to the subject a therapeutically effective amount of at least one GLP-1 agonist and a gastrin compound to produce a sustained beneficial effect.

25. A method of treatment comprising administering to a subject a therapeutically effective amount of at least one GLP-1 agonist in combination with administration of at least one gastrin compound which upon administration to a subject with symptoms of diabetes provides sustained beneficial effects.

26. A method as claimed in claim 25 wherein administration with of at least one GLP-1 agonist in combination with administration of at least one gastrin compound provides sustained beneficial effects of at least one symptom of diabetes.

27. A method as claimed in claim 25 wherein therapeutically effective amounts of the GLP-1 agonist and the gastrin compound are combined prior to administration to the subject.

28. A method as claimed in claim 25 wherein therapeutically effective amounts of the GLP-1 agonist and the gastrin compound are administered to the subject sequentially.

29. A method as claimed in any preceding claim wherein therapeutically effective amounts of a GLP-1 agonist and a gastrin compound are administered at a physiologically acceptable pH.

30. A conjugate comprising a GLP-1 agonist linked to a gastrin compound to provide beneficial effects, in particular sustained beneficial effects.

31. A method of preparing a stable pharmaceutical composition of a GLP-1 agonist comprising mixing a GLP-1 agonist, a gastrin compound, and a pharmaceutically acceptable carrier, excipient, or vehicle effective to physically stabilize the GLP-1 agonist and adapted to provide beneficial effects preferably sustained beneficial effects.

32. A method of treating a condition or disease comprising administering a therapeutically effective amount of a GLP-1 agonist and a gastrin compound, or a composition or conjugate of any preceding claim to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects.

33. A method of treating a condition or disease comprising administering a GLP-1 agonist and a gastrin compound, or a composition or conjugate of any preceding claim with a plurality of cells to a subject in need thereof to thereby produce beneficial effects, preferably sustained beneficial effects.

34. A method for treating a subject with a condition or disease comprising contacting *ex vivo* a plurality of cells with a GLP-1 agonist and a gastrin compound, or a composition or conjugate of any preceding claim, optionally culturing the cells, and administering the cells to the subject in need thereof.

35. A method of any preceding claim wherein the condition or disease is dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, Alzheimer's disease and other central and peripheral neurodegenerative conditions chronic heart failure, fluid retentive states, metabolic syndrome and related diseases, and disorders and obesity.

36. A method for inducing islet neogenesis in a subject comprising contacting islet precursor cells with a GLP-1 agonist and a gastrin compound, or a composition, or conjugate of any preceding claim in a sufficient amount to increase proliferation of islet precursor cells in the subject thereby inducing islet neogenesis.

37. A method for expanding and differentiating stem cells into insulin secreting cells comprising contacting the stem cells with an effective

amount of a GLP-1 agonist and a gastrin compound or a composition or conjugate of any preceding claim.

38. Use of a composition comprising a combination of at least one GLP-1 agonist and at least one gastrin compound for the preparation of a medicament for the treatment of a condition or disease.

39. A use of claim 38 wherein the condition or disease is dyslipidemia, hyperglycemia, severe hypoglycemic episodes, stroke, left ventricular hypertrophy, arrhythmia, bacteraemia, septicaemia, irritable bowel syndrome, functional dyspepsia, diabetes, catabolic changes after surgery, stress induced hyperglycemia, gastric ulcers, myocardial infarction, impaired glucose tolerance, hypertension, Alzheimer's disease and other central and peripheral neurodegenerative conditions chronic heart failure, fluid retentive states, metabolic syndrome and related diseases, and disorders and obesity.

40. A kit form of a composition or conjugate as claimed in any preceding claim.

## ABSTRACT OF THE DISCLOSURE

The invention relates generally to compositions and methods comprising a GLP-1 agonist and a gastrin compound, and uses thereof.

5

Sequence Listing

SEQ ID NO. 1

GLP-1 (1-37):
His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly

SEQ ID NO. 2

GLP-1 (7-36):
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg

SEQ ID NO. 3

Exendin-3 (Heloderma horridum horridum) Genbank Accession No. P20394

1 hsdgtftsdl skqmeeeavr lfiewlkngg pssgappps

SEQ ID NO. 4

Exendin-4 (Heloderma suspectum) Genbank Accession No. HWGH4G

1 hgegtftsdl skqmeeeavr lfiewlkngg pssgappps

SEQ ID NO. 5

N-terminal Glp-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

SEQ ID NO. 6

N-terminal Glp-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe

SEQ ID NO. 7

N-terminal Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe

SEQ ID NO. 8

N-terminal Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Leu-Asp-Phe

SEQ ID NO. 9

mqrlcvyvli falalaafse aswkprsqqp daplgtganr dlelpwleqq gpashhrrql gpqgpphlva dpskkqgpwl eeeeeaygwm dfgrrsaede n

SEQ ID NO. 10
GLP-1 (7-37):

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly